Reply to reviewers

Reviewer #1: The review deals with an interesting theme. The authors depicted the most affected intestinal area by Crohn's disease, which is the distal ileum, and summarized the main factors that may be involved in the explanation of the disease occurrence. The authors included the most recent and relevant studies from the scientific literature. The structure of the article was well performed, and the English editing is adequate. However, the gaps in the literature about this issue that still remain were not clearly discussed.

We thank you for your comments. We now considered your remarks by adding the following sentences:

(in the paragraph on the enteric nervous system) To that extend, although functional evidence are lacking in the literature to fully support this hypothesis, the increased transit time observed in CD patients could be seen as a consequence of ultrastructural injuries of interstitial cells of Cajal in the myenteric plexus [35,36]. […] The importance of these findings on the natural history of CD remains to be determined though. Especially, the effect of the modulation of the ENS in the neuro-immune interplay needs to be investigated.

(at the end of the paragraph on bile acids) Nevertheless, there is only limited evidence on the therapeutic role of bile acids in ileal CD. If several authors reported the protective role of ursodeoxycholic acid and of its precursor, the lithocholic acid, against chemically induced colitis in mice[80–82], there is a lack of data about the effect of an oral supplementation in ursodeoxycholic acid in patients with IBD. Thus, only one small single-centre trial examined the effect of ursodeoxycholic acid on UC and none was performed in patients with CD [83].

(in the section about AIEC) As highlighted by a recently published article, factors influencing the ileal susceptibility to AIEC are numerous and among them epigenetic regulators also have a modulating activity on a large range of proteins[95]. For this reason, before considering a clinical application in ileal CD, further studies are needed to prevent from possible adverse effects induced by the modulation of these epigenetic targets or to identify more specific genes associated with AIEC colonization. Another promising approach would be the use of bacteriophages to target specifically AIEC in ileal disease[96]. Albeit no bacteriophage is approved yet in the EU or in the US for intestinal human therapeutic use, numerous studies reported encouraging results in vivo and therefore data about their effect on the human microbiota are needed in the future[96].

(at the end of the section about diet) As highlighted by this European consensus, good quality data regarding the effects of experimental diets are lacking in the literature. Espacially, randomized control trials are too sparse to recommend a more specific diet in patients with active ileal CD[121].

(in the section entitled "A dedicated treatment of ileal CD?) Interventional works specifically dedicated to the study of the ileal CD treatment are henceforth required.

Figure 1 refers to ileal Crohn's disease, but the drawing in the center of the figure illustrates not only ileal CD but other phenotypes; I suggest the authors amend it. Therefore, the manuscript presents sufficient quality to be published in this journal but needs minor review.

We have taken your remarks into account, and we have made some changes to figure 1. Especially, the drawing in the center of the scheme was changed to illustrate specifically the ileal Crohn's disease.

Best regards

Reviewer #2: I have finished the revision of the Manuscript Number: Manuscript ID: 82831 Manuscript Title: Crohn's disease : why ileum ? All Author List: Nicolas Richars, Guillaume Savoye, Mathilde Leboutte, Asma Amamou, Subrata Ghosh and Rachel Marion-Letellier Manuscript Type: Review In the manuscript the authors described that the ileum is frequently affected by Crohn's disease (CD). Ileal CD differs from other CD types in its epidemiology and natural history. Anatomical and histological features of the ileum provide the keys to understanding this singular disease. Moreover, we herein discuss the crosstalk that takes place in the ileum between an individual and his environment and the clinical significance. The review is very interesting but I have some notes.
#1 To strengthen the review I suggest the authors have a topic on histological changes in the ileum affected by Crohn's disease (CD).

We thank you for your help in improving our manuscript. We added the following paragraph to precise the histological changes in ileal CD:

From a clinical point of view, most of histological features encountered in ileal CD are also found in other disease like backwash ileitis in UC for instance[29]. Albeit epithelioid granuloma is considered as the histological hallmark for the diagnosis of ileal CD, it is not a prerequisite[30]. In about one quarter of patients with ileal CD, pyloric gland metaplasia resulting from the expression of mucins genes normally specific to the stomach (MUC5AC and MUC6) can be noted[30,31]. Although numerous other histological features are described in ileal CD, focal crypt irregularities is considered by expert consensus as one of the most reliable sign for CD[30].
From a biological point of view, the most noteworthy change is found in Peyer's patches. Peyer's patches are ileal immune structures characterized by a B-cell germinal center surrounded by a T-cell interfollicular region. These mucosal associated lymphoid tissues can be as "gateways" of the intestine. The epithelium under which the lymphoid follicle is located differs from the surrounding villus epithelium of the ileum. Indeed, the function of this follicle associated epithelium consists in sampling and transporting luminal antigens through M cells and dendritic cells to CD4+ cells[32]. Early histological changes in Peyer's patches are reported in ileal CD such as an increase of mast cells or erosive epithelial lesions [33,34]. Further, the increased number of glial cells in the Peyer's patches of patients with ileal CD results in an enhanced intestinal permeability[34]. All together these phenomena may explain the increased vulnerability of the ileal mucosa to bacterial invasion in CD patients [35].

Besides, we added a precision about the effect of biological treatment on the bile salts profile. (in the section dedicated to bile acids) Further, secondary bile acids levels in patients with inflammation limited to the ileum tend to increase after biological treatment reaching a similar level with control subjects[75].

#2 I suggest the authors have another topic which describe the effects of the Crohn's disease (CD) on the enteric nervous system of the ileum, such as a decrease in neurons in the myenteric and submucosal plexuses, changes in motility in the ileum.

We appreciate your suggestion that improves the originality of our manuscript. Therefore, we follow your advice and add the following section:

As mentioned in the previous section, histological changes observed in ileal CD include glial cells and the enteric nervous system (ENS). In patients with ileal CD, both the submucous and the myenteric plexus present an overall increase in the number of neuronal cell bodies, enteroglia and interstitial cells of Cajal associated with an upregulation of apoptosis in enteric neurons and enteric glial cells[36,37]. To that extend, although functional evidence are lacking in the literature to fully support this hypothesis, the increased transit time observed in CD patients could be seen as a consequence of ultrastructural injuries of interstitial cells of Cajal in the myenteric plexus [38,39].
Beyond the role of the ENS on intestinal mobility, the density of enteric glial cells conveys a higher risk of ileal CD recurrence after surgery. Thus, after ileocolonic resection for CD, the

inflammation in or around nerve bundles or enteric ganglia is reported by several clinical studies as a risk factor for CD recurrence[40–43]. In the uninflamed section from ileocolonic samples, the number of S100-positive enteric glial cells was enhanced in patients with a relapsing disease unlike vasoactive intestinal polypeptide or substance P positive cells[44]. Furthermore, the ileum of CD patients harbours a different distribution of enteric glial cells with a higher density of these cells around Peyer's patches. Parallelly, the mediators of enteric glial cell increase the permeability of the ileal mucosa in CD patients whereas they decrease the permeability of the mucosa in non-IBD patients[34]. The importance of these findings on the natural history of CD remains to be determined though. Especially, the effect of the modulation of the ENS in the neuro-immune interplay needs to be investigated.

#3 Figures 1 and 2 are fine, however they need to be explained better in the captions.

We re-write the captions of figure 1 and 2:
Figure 1: While Crohn's disease (CD) can be found in any part of the gastrointestinal tract, the ileum is frequently affected. Potential factors involved in ileal CD are genetic susceptibility genes and most of these genes are associated with Paneth cell dysfunction. Environmental factors may be also suspected such as diet, gut microbiota and smoking. Diet can modulate the composition of bile acids along with the microbiota which in return affect the ileal susceptibility to inflammation.

Figure 2: Paneth cells are secretory epithelial cells located in the intestinal crypts. Paneth cells produce antimicrobial peptides in response to bacterial components and support stem cells function through Wnt signaling. Most of the susceptibility genes associated with ileal CD involved a Paneth cell dysfunction. NOD2 and leucine-rich repeat kinase 2 (LRRK2) gene are expressed in Paneth cells and their deficiencies in ileal CD modulate the expression of antimicrobial peptides such as alpha-defensins or lysosyme. Similarly, the reduction of the Wnt-signalling pathway transcription factor Tcf-4 is associated with ileal CD predisposition and leads to a reduced expression of Paneth cell defensin expression. Similarly, blocking of calcium-activated potassium channel protein (KCNN4) inhibits mouse Paneth cell secretion in response to bacterial stimulation.

Best regards,

Dr Rachel Marion-Letellier